CALIFORNIA DEPARTMENT OF FOOD AND AGRICULTURE
SUMMARY OF TOXICOLOGICAL DATA
TOXICOLOGY BRANCH

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CHLORAMBEN

SB 950-029, Tolerance # 266

July 28, 1986
Revised July 1 , 1987

I. DATA GAP STATUS

Chronic rat: Data gap, inadequate study, no adverse effect indicated.

Chronic dog: Data gap, inadequate study, no adverse effect indicated.

Onco rat: Data gap, inadequate study, no adverse effect indicated.

Onco mouse: Data gap, inadequate studies, possible adverse effect indicated.

Repro rat: Data gap, inadequate study, no adverse effect indicated.

Terato rat: Data gap, inadequate study, no adverse effect indicated.

Terato rabbit: Data gap, inadequate study, possible adverse effect indicated. Repeat study in progress.

Gene mutation: No data gap, possible adverse effect.

Chromosome: No data gap, possible adverse effect

DNA damage: No data gap, no adverse effect.

Neurotox: Not required at this time.

Note, Toxicology one-liners are attached

** indicates acceptable study
Bold face indicates possible adverse effect
Reviews by F. Martz, J. Schreider and J. Gee
File name SB029CHL.JG1, Toxicology Summary by J. Gee

The chemical grouping includes chloramben acid and sodium salt (SB950-029) and the ammonium salt (SB950-561). All studies were conducted with either the free acid or the sodium salt (SB 950-029). Tolerance numbers are 266 and 50689. The studies are filed under #266.

CALIFORNIA DEPARTMENT OF FOOD AND AGRICULTURE
SUMMARY OF TOXICOLOGICAL DATA
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CHLORAMBEN, AMMONIUM SALT

SB 950-561, Tolerance # 266

July 28, 1986
Revised July 1 , 1987

I. DATA GAP STATUS

Chronic rat: Data gap, inadequate study, no adverse effect indicated.

Chronic dog: Data gap, inadequate study, no adverse effect indicated.

Onco rat: Data gap, inadequate study, no adverse effect indicated.

Onco mouse: Data gap, inadequate studies, possible adverse effect indicated.

Repro rat: Data gap, inadequate study, no adverse effect indicated.

Terato rat: Data gap, inadequate study, no adverse effect indicated.

Terato rabbit: Data gap, inadequate study, possible adverse effect indicated. Repeat study in progress.

Gene mutation: No data gap, possible adverse effect.

Chromosome: No data gap, possible adverse effect

DNA damage: No data gap, no adverse effect.

Neurotox: Not required at this time.

Note, Toxicology one-liners are attached

** indicates acceptable study
Bold face indicates possible adverse effect
Reviews by F. Martz, J. Schreider and J. Gee
File name SB029CHL.JG1, Toxicology Summary by J. Gee

The chemical grouping includes chloramben acid and sodium salt (SB950-029) and the ammonium salt (SB950-561). All studies were conducted with either the free acid or the sodium salt (SB 950 - 029)-. Tolerance numbers are 266 and 50689. The studies are filed under #266.

II. TOXICOLOGY SUMMARY

COMBINED (CHRONIC + ONCO), RAT

014 37007 "Two-year Carcinogenesis Study in Rats: Technical Chloramben: Final Report." (5/79, Litton Bionetics, project 20576) Chloramben, (89% sodium chloramben, 21-BAC-63); tested at 0, 100, 1000, or 10,000 ppm in the diet for two years; 55/sex/group; NOEL: tentatively 10,000 ppm; no chronic or oncogenic effects reported;unacceptable (dose levels not high enough and not justified; body weight and food consumption recorded on only 50% of animals; hematology done on only 5/sex/group with no 18 month bleeding; no indication that clinical observations were recorded; brain weights were not recorded; histopathology is incomplete, no analyses of diets, not upgradeable. Martz, 12/13/85
EPA 1-liner: No CORE grade. Systemic NOEL > 10,000 ppm (HDT), oncogenic NOEL > 10,000 ppm.

015, 016 37008, 37009 Addenda to 37007. Pathology evaluation and individual data.

020 51001 Stability of the free acid in water as a function of pH, temperature and time.

020 50997 Purity of sodium chloramben lots.

CHRONIC, RAT

002 926055 "Two-Year Dietary Feeding - Rats." (5/15/63, Hazleton Labs) Chloramben (97%, Amiben); fed at 0, 100, 1000, or 10,000 ppm in the diet for two years; 35/sex/group in treatment groups, 70/sex/group in controls; NOEL: tentatively 10,000 ppm; unacceptable, not upgradeable (wide weight variation, inadequate high dose, only 5/sex in control and high dose for histopathology, inadequate survival from intercurrent respiratory disease, no analysis of diets for actual content.) No adverse effect identified.
Schreider, 3/5/85.
EPA 1-liner: No CORE grade. Systemic NOEL = 1000 ppm in diet (50 mg/kg), oncogenic NOEL > 10,000 ppm (HDT).

013 37004 Exact duplicate of 926055.

013 37005, 37006 Addenda (histopathology) to 926055.

020 50999 Duplicate of 926055 plus addenda of individual histopathology for 52-week sacrifice and terminal sacrifice.

CHRONIC, DOG

002 926056 "Two-year Dietary Feeding - Dogs." (6/10/63, Hazleton) Chloramben (97%, Amiben); fed at 0, 100, 1000, 10,000 ppm in the diet; 4/sex/group; NOEL: tentatively 10,000 ppm; unacceptable (major variances from guidelines), not upgradeable (no diet analysis, inadequate histopathology tissues, no age given). No test article-related adverse effect identified.
Schreider, 3/5/85.
EPA 1-liner: Minimum. Systemic NOEL > 10,000 ppm in diet (250 mg/kg).

013 37003 Exact duplicate of 920056.

ONCOGENICITY, RAT

006 926059 "Bioassay of Chloramben for Possible Carcinogenicity." (9/77, Gulf South Res. Inst. for NCI, Report no. 25) Chloramben, 90-95% technical grade Amiben; fifty/sex/group fed at 0, 10,000 or 20,000 ppm for 80 weeks and observed an additional 33 weeks; 10/sex for control group. Major variances from guidelines; unacceptable, not upgradeable (no hematology, no food consumption or individual body weights, animals missing, two doses only, insufficient length of exposure to test material. No adverse oncogenic effect reported. Schreider, 3/7/85
No EPA 1-liner.

ONCOGENICITY, MOUSE

006 38193 "Bioassay of Chloramben for Possible Carcinogenicity." (9/77, Gulf South Res. Inst. for NCI, Report no. 25) Chloramben 90-95%, technical grade Amiben; B6C3F1 mice; 50/sex/test group, 10/sex for controls; fed 0, 10,000 or 20,000 ppm, 80 week exposure; diets were analyzed periodically (Appendix G, mean value only); unacceptable, (inadequate concurrent controls, no food consumption, individual animal weights, dose selection, animals housed in the same room as other studies using pesticides). An adverse oncogenic effect was reported in males and females at 20,000 ppm for hepatocellular carcinoma. Also, a marginal effect in females at 10,000

ppm. See EPA registration standard (1981) Document 266-004 for a discussion of these results. Schreider, 3/7/85.
EPA 1-liner: Supplementary. Systemic NOEL = 20,000 ppm (HDT).

017 37010 "Eighteen (18) Month Oncogenic Study Following Prolonged Oral Administration in CRL:COBS CD-1 Mice of Amiben Acid Technical." (10/20/ 78, Huntingdon, HRC 1-362) Chloramben, technical, lot 1004-132 (90% - see # 50997); 50/sex/group were fed 0, 100, 1000, or 10,000 ppm for 18 months; NOEL: <100 ppm, (liver changes, not onco) >10,000 ppm; (clinical obs) various microscopic changes in the liver especially of males are reported as due to enzyme induction and toxicity, but not oncogenic; unacceptable (need diet analysis, individual data on body weights, justification of doses despite positive findings), possibly upgradeable. Gee, 4/23/86.
EPA 1-liner: Supplementary. No conclusion has been made regarding the oncogenic potential of the test material.

018 37011 Addendum to 37010.

020 50997 Purity of chloramben sodium, 1004-132.

REPRODUCTION, RAT

011 36995 "Reproduction Study in Albino Rats with Amchem Products, Inc., Amiben (3-amino-2,5-dichlorobenzoic acid.)" (8/2/66, A.M.E. Assoc.) Chloramben, 90% (Amiben acid); fed at 0, 500, 1500, or 4500 ppm in the diet; 8 males and 16 females per group; 3 generations, 2 litters each; NOEL: not established as doses all too low; no reproductive effects were reported; unacceptable (no justification of dose selection and no evidence that MTD was achieved, no diet analysis, no histopathology on adult breeders, no individual data), not upgradeable. Gee, 4/23/86.

EPA 1-liner: No CORE grade. Systemic NOEL > 4,500 ppm (HDT), reproductive NOEL > 4,500 ppm.

TERATOGENICITY, RAT

011 36994 "Teratology Study in Rats: Technical Chloramben: Final Report." (4/20/76, Litton Bionetics, project 2577) Chloramben, technical Amiben, lot 1004-132, 83%; in the diet at 0, 500, 1500, or 4500 ppm on days 6-15 of gestation; NOEL: 500 ppm is claimed, but cannot be accepted because actual dose received is not verifiable; slight increase in embryomortality and reduced skeletal development ocurred at the high dose, while only skeletal development effects ocurred at the intermediate dose; unacceptable (MTD not achieved and no analysis of dosing material), not upgradeable. Martz, 12/12/85 and J. Gee, 6/25/87. EPA 1-liner: Minimum. Teratogenic NOEL ≥ 4500 ppm (HDT), fetotoxic NOEL = 500 ppm (reduced ossification of skeletal bones), maternal NOEL ≥ 4500 ppm (HDT).

020 51001 Stability of chloramben free acid in water.

TERATOGENICITY, RABBIT

011 36993 "Teratology Study of Chloramben Sodium Salt in New Zealand White Rabbits." (4/6/84, Science Applications, UNC/SAI 1282018) Chloramben sodium salt (83%, RFM2846AC); by oral gavage at 0, 200, 333 and 500 mg/kg on days 6-18 of gestation by gavage; NOEL: tentative fetotoxicity 333

mg/kg(nominal); slight fetotoxicity and intrauterine growth retardation occurred at the high dose level only; unacceptable (dosing levels cannot be verified and data integrity questionable), not upgradeable. Record # 51000 in document 020 contains the weighing records with the notations that the actual doses were not 0, 250 500 and 1000 mg/kg as stated throughout the report but 0, 200, 333 and 500 mg/kg. The rebuttal states that following the procedure in # 51000 (Document 266-020), the true dosages were 0, 215, 390.6 and the high dose was incompletely soluble. Martz, 12/13/85 and Gee, 6/25/87.

020 Repeat rabbit teratology study is in progress in 1987.

MUTAGENICITY, GNMU

Microbial Systems

012 36997 "Ames Salmonella/Microsome Plate Test with and without Metabolic Activation on: Sodium Amiben 21-BAC-63." (11/27/78, Pharmakon Labs) Chloramben, sodium salt (Amiben, 21-BAC-63, 89% from # 50997 in Document 266-020); Salmonella strains TA 1537, TA1535, TA1538, TA98, and TA100 tested +/- S9 with chloramben at 0, 40, 200, 1000, 5000, or 50,000 ug/plate in duplicate , 1 trial; no increase in reversion rate reported, some cytotoxicity at 50,000 ug/plate; unacceptable (no repeat trial, mix for S9 not described, positive controls not present for all conditions), not upgradeable. Gee, 4/22/86.

012 37001 "Mutagenicity Evaluation of Chloramben Sodium Salt in the Ames Salmonella/microsome Plate Test: Final Report." (1/21/85, Litton Bionetics, 10.

Project no. 20988) Chloramben, sodium salt; Salmonella strains TA97, TA100, TA1535, TA1537 and TA1538 tested +/- S9 with chloramben at 1, 10, 100, 50, 1000, 2500, 5000, 10,000 ug/plate; 1 plate, 1 trial; no evidence of increased reversion at any level; unacceptable (no replicates, no repeat trials), not upgradeable. Gee, 4/23/86.

SUMMARY: While neither study alone fulfills the requirement due primarily to the lack of an independent, confirming trial, collectively they contain sufficient data to determine that chloramben is not mutagenic in this strain of bacteria.

Mammalian Cells

**** 012 36998** "Mutagenicity Evaluation of Chloramben Sodium Salt in the Mouse Lymphoma Forward Mutation Assay: Final Report." (4/29/82, Litton Bionetics, project no. 20989) Chloramben tested on mouse lymphoma cells (L5178Y) at 0, 125, 250, 500, 100, and 2000 ug/ml +S9; 0, 2000, 4000, and 6000 ug/ml without S9; 4 hour exposure, 2 day expression; 2 trials; increase in mutation frequency at 2000 ug/plate +S9 only; no clear dose response; acceptable but low cloning efficiency, EMS control showed no increase in mutation frequency and wide scatter in experimental values in trial 1 and some plates lost to contamination with DMN controls +S9 below historical values given in the text in trial 2. Initially reviewed as unacceptable due to problems as described above. Upgraded to acceptable with an adverse effect in view of the registrant’s rebuttal and the positive effect.
Gee, 4/23/86 and 6/26/87..

MUTAGENICITY, CHROMOSOME

**** 012 37000** "Mutagenicity Evaluation of Chloramben, Sodium Salt in an in vitro Cytogenetic Assay Measuring Chromosome Aberration Frequencies in Chinese Hamster Ovary (CHO) Cells: Final Report." (3/23/82, Litton Bionetics, project no. 20990) Chloramben tested on Chinese hamster cells (CHO-WBI) at 10 ug/ml to 25 mg/ml +/-S9; +S9 2 hour exposure, -S9 8.5 to 10 hour exposure; 3 trials each; positive for aberrations; acceptable. From the statements in the text, it appears that the positive findings are at concentrations showing significant toxicity. Initially reviewed as unacceptable due to lack of clear presentation of the cytotoxicity. In view of the discussion in the rebuttal, Document 266-020, Tab D, the study is upgraded. Gee, 4/23/86 and 6/26/87.

**012 37002 "Clastogenic Evaluation of Chloramben in the Mouse Bone Marrow Cytogenetic Assay: Final Report." (5/22/85, Litton Bionetics, project no. 22202) Chloramben, (87%) tested at 0, 0.1, 0.33, or 1.0 g/Kg i.p. in a single dose or 5 i.p. injections of 0.75, 0.25, or 0.075 g/Kg; 5 mice/sex/group; sampled at 6, 24,or 48 hrs after single injection, 6 hrs after multiple; no aberration increase due to treatment; ACCEPTABLE. These results differ from the findings in 37000 above. The reasons may be route of administration and non-penetration of the compound to the bone marrow because no marrow cytotoxicity is reported or metabolism by the whole animal detoxifies. Also, the in vitro test above showed effects in the presence of significant cytotoxicity. For in vivo chromosomal aberrations study, guidelines specify that either an mtd or evidence of cytotoxicity be demonstrated. The report contains results of two acute toxicity studies and based on the mortality at 2 mg/kg, the doses selected for this study seem

reasonable. However, no mortality or clinical toxicity are reported here. Gee, 5/5/86.

SUMMARY: A recent publication by E. D. Thompson in Mutation Research 8: 753 (1986), compared the results in vitro for cytogenetics with in vivo results for cytogenetics or micronucleus formation for 216 chemicals. He concluded that 97% of the in vivo clastagens are positive in vitro but that the in vitro tests have a high incidence of "false positives" and a positive effect should be confirmed in animals studies. The author, however, did not use the stringent criteria used by EPA's Gene-Tox reports so the adequacy of each the studies was not addressed. In the case of chloramben, an acceptable in vivo test is on file. There is no assurance, however, that the bone marrow is a target tissue while there is evidence that the liver is one. In addition, although the in vivo study was evaluated as acceptable based on justification of the dose by mortality in acute studies, no clinical signs were reported in the actual test. For these reasons, the in vitro findings should be considered in the evaluation of the possible toxic effects. Gee, 6/26/87.

MUTAGENICITY, DNA/OTHER

012 36996 "Summary data; Primary DNA Damage, E. coli Plate Test." (12/1/78, Pharmakon) Chloramben, Amiben, 21-BAC-63, 89%; tested at 0, 0.05, 0.5, 5, 50, or 500 mg/ml solutions; +/-S9 on E. coli strains W3110 (A+) and P3478 (pol A^-) in agar plates; 10 ul were applied to 7 mm disks -S9 and 50 ul was added per 8 mm/well +S9; zones of inhibition were comparable; only 500 mg/ml showed growth inhibition; unacceptable (no justification of high dose,

composition of S9 not provided, HAT medium composition not given).
Gee, 4/23/86.

** 012 36999 "Evaluation of Chloramben, sodium Salt in the Primary Rat Hepatocyte Unscheduled DNA Synthesis Assay: Final Report." (3/24/82, Litton Bionetics, project no. 20991) Chloramben tested at 0, 5, 10, 25, 50, 100, 250, 500 and 1000 ug/ml on rat hepatocytes; 150 cells evaluated at each level; exposed for 18 hrs.; no evidence of UDS; 1000 ug/ml cytotoxic; acceptable (individual cell counts are given in # 50998 in document 266-020). Initially reviewed as unacceptable but upgradeable with submission of information on test material and individual data for grain counts. Gee, 4/23/86 and 6/25/87.

NEUROTOXICITY

Not required at this time.